Progress Report

The present report summarizes the research activities conducted during the period of June, 2011 to January, 2012 related to the project entitled "Developing a quick and reliable method to screen potato germplasm and advanced clones for resistance against pink rot disease caused by *Phytophthora erythroseptica* Pethyb" funded by CCPGA.

Collection of Phytophthora erythroseptica isolates: We collected the infected tubers from the fields of SLV with the help of Dr. Rob Davidson to isolate the *Phytophthora erythroseptica* isolates by employing standard isolation methods. Briefly, cut pieces of infected tubers were surface sterilized and placed in Petri plates on top of wet filter paper and incubated under continuous light to induce sporulation. Single spores were transferred to water agar plates and allowed to germinate to obtain pure cultures. In total, seven pure isolates were collected based on the colony morphology and further confirmed by inoculating Russett Norkotah potato tubers; disease assessment was performed by using the method described by Thompson et al. (2007). Among the seven isolates, only one isolate (SLV1) was positive and showed typical pink rot disease symptoms (Fig. 1). In addition to these isolates, there are four other isolates (188-4, 217-1, 266-2 and PE-89) were received from Dr. Thompson, North Dakota State University for comparison purposes. Among these four isolates, PE-89 and 217-1 are mefoxonam (fungicide used to treat pink rot disease) resistant, 266-2 is mefoxonam sensitive, and 188-4 is mefoxonam low intermediate.

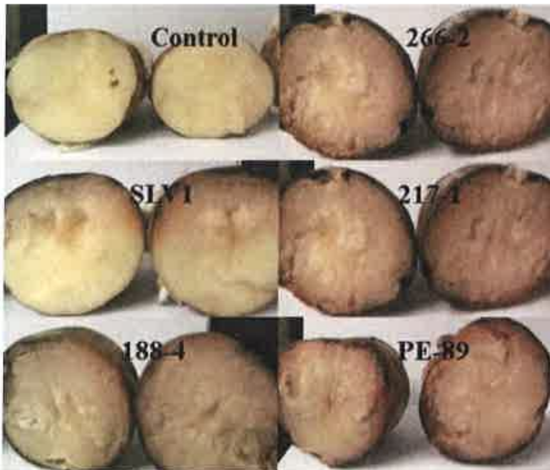

Figure 1. Pathogenicity assay of *P. erythroseptica* isolates on the tubers of Russett Norkotah. Control: mock inoculated; 266-2: mefoxonam sensitive isolate; SLV1: isolated from the fields of SLV; 217-1: mefoxonam resistant isolate; 188-4: mefoxonam low intermediate isolate; PE-89: mefoxonam resistant isolate.

Collection of potato clones: Resistant and susceptible potato clones were obtained from the collection of Dr. Holm, SLVRC and Dr. Thompson, North Dakota State University. We used five clones (Snowden, Russett Norkotah, Atlantic, Etb 6-21-12 and Etb 6-5-2) and developed virus free tissue culture plantlets with the help of Tissue Culture Laboratory, SLVRC. After developing the tissue culture plantlets, the plantlets were moved to Department of Horticulture, CSU for further multiplication and transfer to pots for pathogenicity experiments. Briefly, all tissue culture plantlets derived from all five clones were maintained in the Majenta boxes containing LS agar media and incubated in the growth chamber under continuous light at 25C until transferred to pots. Among these five clones, Russett Norkotah is highly susceptible and Atlantic and Snowden are moderately susceptible to pink rot disease. In contrast, Etb 6-5-2 is highly resistant and Etb 6-21-12 is moderately resistant to pink rot disease.

Pathogenicity experiment: We performed the pathogenicity assay by using three *P. erythroseptica* isolates inoculated independently on five different potato clones under greenhouse conditions at the Plant Growth Facility, CSU. We chose three *P. erythroseptica* isolates: PE-89 (mefoxonam resistant), 266-2 (mefoxonam sensitive) and SLV1 (from SLV). Similarly, we chose five different potato clones: Russett Norkotah (highly susceptible), Snowden and Atlantic (moderately susceptible), Etb 6-5-2 (highly resistant) and Etb 6-21-12 (moderately resistant). Briefly, we grew the plantlets of all five clones under laboratory conditions by employing tissue culture techniques and transferred each plantlet to pots when plantlets were 13 to 15 days old with small roots. All these clones were grown in pots containing regular potting soil in the greenhouse under 25C with 70% humidity (Fig. 2).

Figure 2. Snapshot showing potato plants grown in the greenhouse bench at Plant Growth Facility Center at CSU.

When plants were 5-6 weeks old, we inoculated all clones with three *P. erythroseptica* isolates independently following the method described by Thompson et al. (2007). For each isolate and each clone we inoculated three to four plants and collected the root tissues and tubers at two time points: one week and three weeks after inoculation (Fig. 3). Triplicates were maintained for each isolate inoculated with each clone; mock inoculated plants were considered controls for each clone. Root tissues and tubers were collected at one week and three weeks after inoculation. All root tissues and tubers were stored at -80C for further proteomic analyses. Based on our pathogenicity assays the clones Russett Norkotah,. Atlantic and Snowden showed highly susceptible and Etb-6-5-2 showed resistance to all three isolates tested in this study (Fig. 3).

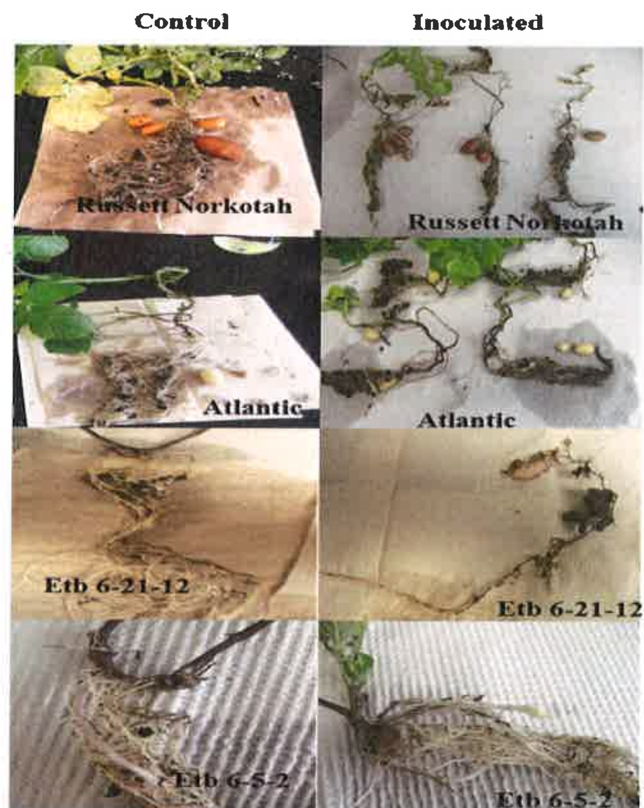

Figure 3. Picture showing the representative potato plants of each clone infected with *P. erythroseptica* isolate. Control: mock inoculated; Inoculated: inoculated with the fungus. The picture was taken three weeks after fungal inoculation.

Proteomic analyses: Currently, we are extracting total proteins from the infected root tissues and will perform one dimensional SDS-PAGE to visualize the differences in their protein profiles compared with the controls. Based on this result, we will further process the samples which show differences consistently between the clones infected with three different *P. erthroseptica* isolates by proteomic analyses employing 2D SDS-PAGE electrophoresis using the method described by De-la-Pena et al. (2008). We anticipate that this method will allow us to identify proteins unique to the resistant cultivars that may serve as biomarkers for high throughput screening such as ELISA.

References:

Thompson, A.L., Taylor, R.J., Pasche, J.S., Novy, R.G. and Gudmestad, N.C. (2007) Resistance to *Phytophthora erythroseptica* and *Pythium ultimum* in a potato clone derived from *S. berthaultii* and *S. etuberosum*. *American Journal of Potato Research* **84:** 149-160.

De-la-Pena, C., Lei, Z., Watson, B.S., Sumner, L.W. and Vivanco J.M. (2008) Root-microbe communication through protein secretion. *Journal of Biological Chemistry* **283:** 25247-25255.